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Smoking Technique

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

Date: October 1, 1954

1. Name of Investigator: O. W. H. Schepers, M.D., D.Sc.

2. Title: Director

3. Institution & Address: The Saranac Laboratory
7 Church Street
Saranac Lake, New York

4. Project or Subject:

ENVIRONMENTAL PULMONARY CARCINOGENESIS. The co-carcinogenic potentialities of inhaled tobacco smoke in relation to beryllium-provoked lung cancer of the rat.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

(a) See attached explanatory memorandum.
(b) The Saranac Laboratory has perfected a technique of producing lung cancer in rats by means of beryllium aerosol inhalation.
(c) It is proposed to examine the co-carcinogenicity of tobacco smoke with beryllium sulphate in rats.
(d) The incidence and characteristics of pulmonary carcinoma will be studied in 500 rats which will be subjected to detailed histological and biochemical analysis.
(e) Special precautions need to be taken to safeguard personnel against beryllium toxicity.

Signature
Director of Project

O. W. H. Schepers, M.D., D.Sc.
Director, The Saranac Laboratory
and as
Executive Secretary of the Saranac Laboratory Board of

1003541029

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

6. Budget Plan:

2 years

Salaries	$21,120.
Expendable Supplies	14,465
Permanent Equipment	3,500.
Overhead	9,771.
Other	500.
Total	$49,356

Date: October 1, [illegible]

7. Anticipated Duration of Work: G. W. Schepers, M.D., D.Sc.

24 months

8. Facilities and Staff Available:

See attached memorandum

The Saranac Laboratory
7 Church Street
Saranac Lake, New York

9. Additional Requirements:

A liberal gratis source of supply of tobacco in the form in which it is currently being consumed by the public.

10. Additional Information (Including relation of work to other projects and other sources of supply):

(a) See attached memorandum.
(b) The work will be based on and amplify studies which have been conducted by The Saranac Laboratory during the past 4 years by means of grants-in-aid from The Damon Runyon Memorial Fund for Cancer Research, The American Cancer Society, The U. S. Atomic Energy Commission
(c) [illegible]
(d) Special precautions need to be taken to safeguard personnel against beryllium toxicity.

Signature _____
Director of Project

G. W. H. Schepers, M.D., D.Sc.
Director, The Saranac Laboratory
and as
Executive Vice-President of The Saranac Laboratory Board of Trustees
Business Officer of the Institution

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I. RATIONALE

The Saranac Laboratory has pioneered research into the problems of chest diseases during the past seventy years. During the past thirty years it has concentrated more particularly on the industrial diseases. The long range perspective gained by the eleven hundred experiments already conducted by the Laboratory and the innumerable clinical diagnostic problems it has encountered have brought into sharp focus the growing problem of lung cancer. The Laboratory has, moreover, through its extensive surveys of industrial environmental hazards, become acutely conscious of the question whether the growing incidence of lung cancer does indeed bear some specific causal relationship to the multiplicity of nocuous substances which form part of the respiratory milieu of the civilized human being.

In an attempt to provide a positive answer for some of the questions relating to environmental pulmonary carcinogenesis the Laboratory has conducted statistical surveys to probe the relationship between specific industrial exposures and lung cancer and has conducted a series of experiments in an effort to produce proof for or against the contention that at least some of the foreign substances inhaled by industrial communities play a part in the origin of the growing numbers of neoplasmata.

As a result of this investigation the Laboratory has discovered that a pulmonary adenocarcinoma can be provoked with a high measure of certainty in rats by exposing them for approximately six months to an aerosol of beryllium sulphate. The tumors commence to appear after an induction period of about nine months, and about fourteen months after the onset of the period of exposure there is a sudden progressive peak incidence of carcinomata in various stages of development. There may be as many as three tumors simultaneously in a single animal. This induction period and the total incidence of tumors remain the same no matter whether the beryllium exposure be continued daily for the whole period or whether it be discontinued after an effective dosage has been administered over the first six months.

The cytological components of these tumors betray marked anaplastic phenomena. Metastases have been observed in lymph glands, pleura, kidneys, and other organs studied. On transplantation to the subcutaneous tissues of young rats these lung tumors also survive excellently and continue to proliferate in the manner of true metastases. Two basic types of neoplasmata have so far been identified, namely, an alveolar and a squamous cell type.

Further information which is pertinent to this whole question refers to the fact that the beryllium does not undergo any alteration in the lung tissue and may therefore be recovered from the lungs of rats and thus be quantitatively correlated with the occurrence of lung cancer. Furthermore, it has thus far been possible to show that neither quartz nor iron-oxide dust inhalation has any influence in facilitating the onset of the cancers.

i.e. they do not act as co-carcinogens. The role of asbestos in this connection is in the process of being explored.

It is impossible to avoid observing the hue and cry which has been set afoot over the possibility that the inhalation of tobacco smoke may serve as an explanation for the rising incidence of lung cancer. The statistical correlation between smoking habits and lung cancer incidences is no doubt somewhat suggestive and the demonstration that tobacco tars contain carcinogenic fractions compels one to consider the theory seriously. Immediately, the following questions come to mind: Why do these carcinogens only act on certain individuals and particularly on males rather than females? This may not merely be a question of endocrines or intensity or duration of their smoking habits but may well be related to the fact that men are more commonly exposed to environmental respiratory hazards of a variety of kinds. Why also do the carcinogens act more strongly on the lung than on the more proximal portions of the respiratory tract where these potential carcinogens should have the greatest gradient of concentration? What factor determines that a cancer shall arise at any particular locality in the lung? If smoke is the cause, why does it produce a single lesion instead of multiple neoplasmata? Having shown that tobacco tar contains carcinogens, has one disproved that other carcinogenic agents derived from the environment, for instance in the air breathed by the smoker or breathed in the process of smoking and altered or concentrated through that process may not be potent factors in the production of these cancers? Our statistics have not adequately differentiated between the incidence of lung cancer in industrial versus non-industrial populations.

A higher probability appears to be that before the potential carcinogenic substances in tobacco smoke can have any specific influence in provoking abnormal cell growth they must be implanted in a ready prepared tissue bed where they would operate synergistically with other substances. Such co-carcinogens may be metabolic products deriving from the host tissue e.g., endocrine derivatives, or they may have been introduced into the lungs from the environment. It is the current consensus of opinion that the neoplasm phenomenon thus has its origin in multiple causes.

It is now proposed to use the beryllium method in relation to tobacco smoke. By rendering lung tissue cells unstable through prior exposure to beryllium the addition of a second carcinogenic agent ought to precipitate lung tumors at a greater rate and sooner than would at present be anticipated for beryllium alone. It is proposed to use tobacco smoke at the secondary potential co-carcinogen.

While not wishing to deny entirely the merits of experiments which have already been extensively conducted on tumor-susceptible mice, it must be pointed out that when one starts off with an unknown primary carcinogen, or series of carcinogens as in these mice, it becomes extremely difficult to interpret the effect of a substance under test, e.g., tobacco tars, while there is no known method of quantitating these unknown primary co-carcinogens. The beryllium method applied to the rat not only has the advantage

that the beryllium actually deposited in the rat lung can be quantit tively determined by means of methods perfected in our biochemistry department, but it must also be emphasized that the tumor to be provoked is one which does not occur naturally in these rats so that it is a specific lesion provoked by a known substance and whose statistical incidence bears a calculable relationship to the amount of beryllium retained in the lung tissue of the experimental animal. Provided, therefore, that these factors are properly controlled as they may be, any enhanced or earlier incidence of tumors in animals exposed to tobacco smoke would signify a synergistic relationship between them. Should the tobacco smoke, on the other hand, retard the onset of tumors or diminish the total incidence of these, one might be able to infer an anticarcinogenic effect.

II. DETAILED PLAN OF PROCEDURE

A. 250 rats will be exposed to an aerosol comprising a known constant concentration of beryllium sulphate dust introduced into the atmosphere of the experimental chambers for eight hours each day, 5 days per week for a total period of six months. It is anticipated that a number not exceeding about fifty of these rats may succumb to acute beryllium toxicity.
   (a) Half of the surviving rats will be daily exposed to freshly produced tobacco smoke for an additional period of six months.
   (b) The remaining half will be transferred to fresh air to serve as a control.

B. The experimental procedure will be reversed by exposing 400 rats to inhalation of fresh tobacco smoke for six months and thereafter dividing the survivors into three groups, viz:
   (a) Half of the survivors to undergo exposure to the beryllium sulphate aerosol.
   (b) A quarter to revert to residence in clean air.
   (c) The remainder to continue exposure to the smoke chamber.

C. 50 rats of the same age and strain to be kept in normal air throughout the duration of the study as a further control to A and B.

III. X-RAY CONTROL

At monthly intervals sample animal batches from each group (except those currently in the beryllium chambers) will be subjected to macroradiography as the onset of lung tumors can thus be well controlled. This method will determine or modify the fate of individual animals.

IV. SACRIFICING SCHEDULE

Animals of groups A and B will be sacrificed in sample batches at 3, 6, 9, 10, 11, 12, 13, 14, 15 months after the commencement of exposure to either tobacco smoke or beryllium sulphate dust. Any suspect animals discovered by radiography will be similarly sacrificed. All animals will be subjected to histological study and biochemical assays for beryllium will be run on representative samples and organs.

V. PRECAUTIONS AGAINST BERYLLIUM

Certain human individuals have shown an exaggerated susceptibility to the toxic effects of beryllium compounds. It is therefore necessary to conduct the experiments by means of beryllium with elaborate circumspection. Special equipment and methods have been elaborated in The Saranac Laboratory during the past fourteen years to ensure absolute safety for the personnel and the public. This, however, causes considerable expense. The Laboratory has the advantage of having a great deal of the basic equipment, including four special chambers, each with the capacity for 100 animals, to house all the experimental animals for the projected experiment. One new cage will have to be constructed, besides special apparatus to create the tobacco smoke.

VI. BUDGET

A. Personnel and Salaries

PERSONNEL	No.	SALARIES $-Hourly	1st Yr.	2nd Yr.	Total
Pathologists	3	4.00	$1,500.	$3,000.	$4,500.
Biochemist	1	3.00	500.	1,000.	1,500.
Chemist	1	3.00	500.	500.	1,000.
Engineer	1	3.00	500.	500.	1,000.
Research Associate	1	3.00	1,000.	1,000.	2,000.
Photographer	1	2.50	500.	500.	1,000.
Histologist	1	2.50	500.	1,000.	1,500.
Technicians:					
Pathology	1	2.00	600.	600.	1,200.
Animal Care	2	2.00	800.	400.	1,200.
Histology	2	2.00	200.	800.	1,000.
Biochemistry	1	2.00	400.	600.	1,000.
Engineering	1	2.00	600.	200.	800.
Mechanic	1	2.00	400.	400.	800.
Typist	1	2.00	200.	500.	700.
Sub-total			$8,200.	$11,000.	$19,200.
Workmen's Compensation and Superannuation Contribution			820.	1,100.	1,920.
Total			$9,020.	$12,100.	$21,120.

B. Expendable Supplies (continued on next page)

VI. BUDGET (Continued)

A. Personnel and Salaries (Brought forward)		$21,120.
B. Expendable Supplies (2 years)		
Animals: Rats - 500 at $2.00	$1,000.	
Maintenance for 5,250 rat/months at $1.00	5,250.	
Insurance against epizootics	2,500.	
Experimental Chambers		
Maintenance of 5 chambers at $1,500. per annum	3,000.	
Supplies:		
Histological	475.	
Photographic	580.	
Radiographic	560.	
Chemical (mainly beryllium)	500.	
Glassware	100.	
Miscellaneous	250.	
Materials for adapting dust chambers	250.	
		$14,465.
C. Equipment (Permanent)		
New generator for macro-radiography unit	$2,000.	
Additional new beryllium chamber	1,000.	
Animal cages (replacements)	250.	
Microphotographic accessories	250.	3,500.
D. Overhead		
At 25%		9,771.
E. Travel		
Contingent fund		500.
GRAND TOTAL FOR TWO YEARS.		$49,356.

Note: (1) Funds requested for the first year $29,356.
It would be appreciated if at least $3,000. could be made available well in advance of any date of commencement preferred by the Committee to enable the construction of special units and the acquisition of special equipment.

(2) The Saranac Laboratory is a non-profit organization incorporated under the State Education Act by the Board of Regents of Albany, New York.

October 1, 1954

G. W. H. Schepers, M.D., D.Sc., F.C.C.P.
Director, The Saranac Laboratory

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